invitrogen™

iBlot™ Dry Blotting System

For dry, electroblotting of proteins from mini, midi, and E-PAGE™ gels

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User Manual

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Product Contents

Types of Products

This manual is supplied with the following products:

Product	Catalog no.
iBlot™ Gel Transfer Device	IB1001 IB1001UK IB1001EU

iBlot™ Gel Transfer Device Contents

The contents of the iBlot™ Gel Transfer Device are listed below:

Component	Quantity
iBlot™ Gel Transfer Device	1 each
Specific Power Cord based on the type of unit ordered (for U.S./Canada/Taiwan/Japan, Europe, or UK)	1 each
Blotting Roller	1 each
De-bubbling Roller	1 each
iBlot™ E-PAGE™ Tab	10

See page vi for specifications and description of the iBlot™ Gel Transfer Device.

Upon Receiving the Instrument

Examine the unit carefully for any damage incurred during transit. File any damage claims with the carrier. The warranty does not cover in-transit damage.

iBlot™ Transfer Stacks

The following iBlot™ Transfer Stacks are available from Invitrogen:

Product	Transfer Membrane	Catalog. No.
iBlot™ Gel Transfer Stacks, **Regular**	Nitrocellulose	IB3010-01
	PVDF	IB4010-01
iBlot™ Gel Transfer Stacks, **Mini**	Nitrocellulose	IB3010-02
	PVDF	IB4010-02

If you ordered the iBlot™ Gel Transfer Stacks, Regular or Mini, you will receive the components listed in the table below. Store the iBlot™ Gel Transfer Stacks at room temperature. For best results, use the transfer stack before the expiration date printed on the package for each stack.

Product	Quantity
The iBlot™ Gel Transfer Stacks, **Regular** contain:	
iBlot™ Cathode Stack, Top	10
iBlot™ Anode Stack, Bottom	10
iBlot™ Disposable Sponge	10
iBlot™ Filter Paper	10
iBlot™ E-PAGE™ Tab	1
The iBlot™ Gel Transfer Stacks, **Mini** contain:	
iBlot™ Cathode Stack, Top	10
iBlot™ Anode Stack, Bottom	10
iBlot™ Disposable Sponge	10
iBlot™ Filter Paper, Mini	10

Product Specifications

iBlot™ Gel Transfer Device Specifications

Dimensions:	37 cm (l) x 20 cm (w) x 11 cm (h)
Weight:	2.3 kg
Electrical Parameters:	100-240 V, 50/60 Hz, 3.3 A
Built-in Features:	Digital display, alarm, light LED
Compatibility:	Suitable for transfer of mini (8 x 8 cm), midi (8 x 13 cm), and E-PAGE™ Gels
iBlot™ Materials:	Polycarbonate, Cycoloy, Acrylic, Gold plated copper, Stainless steel, Plasticized silicone, Aluminum
Operating Temperature:	5-40°C
Blotting Roller:	Delrin roller attached to a stainless steel handle (8.6 cm wide)

The iBlot™ Gel Transfer Device is impervious to alcohol, acid (HCl), alkali (NaOH) but not compatible with acetone, dimethyl sulfoxide, and acetic acid.

The CE mark symbolizes that the product conforms to all applicable European Community provisions for which this marking is required. The iBlot™ Gel Transfer Device complies with the Underwriters Laboratories Inc. regulation and the European Community Safety requirements. Operation of the iBlot™ Gel Transfer Device is subject to the conditions described in this manual.

The protection provided by the equipment may be impaired if the equipment is used in a manner not specified by Invitrogen.

Continued on next page

Product Specifications, Continued

iBlot™ Gel Transfer Stack Specifications

The iBlot™ Gel Transfer Stacks are used with the iBlot™ Gel Transfer Device and are available separately from Invitrogen (page x). For details on the iBlot™ Transfer Stacks, see page 6. The specifications for the iBlot™ Transfer Stacks are listed below:

iBlot™ Cathode Stack, Top	
Top Stack Gel Layer, Regular:	13.6 cm (l) x 8.5 cm (w) x 0.19 cm (thick)
Top Stack Gel Layer, Mini:	8.5 cm (l) x 8.5 cm (w) x 0.19 cm (thick)
Top Stack Gel Layer Composition:	Proprietary
Electrode:	Copper
iBlot™ Anode Stack, Bottom	
Bottom Stack Gel Layer, Regular:	14.1 cm (l) x 8.5 cm (w) x 0.32 cm (thick)
Bottom Stack Gel Layer, Mini:	8.5 cm (l) x 8.5 cm (w) x 0.32 cm (thick)
Bottom Stack Gel Layer Composition:	Proprietary
Electrode:	Copper
Transfer Membrane:	Nitrocellulose (0.2 µm) or PVDF (0.2 µm, low fluorescence)
Plastic Tray:	16.8 cm x 10.3 cm (1.7 cm wide copper contact)
iBlot™ Disposable Sponge	
Dimensions:	15 cm (l) x 9.5 cm (w)
Material:	White Melamine
Metal Contact:	Aluminum
iBlot™ Filter Paper	
Regular Filter Paper:	13.5 cm (l) x 8 cm (l) x 0.04 cm (thick)
Mini Filter Paper:	8 cm (l) x 8 cm (w) x 0.04 cm (thick)

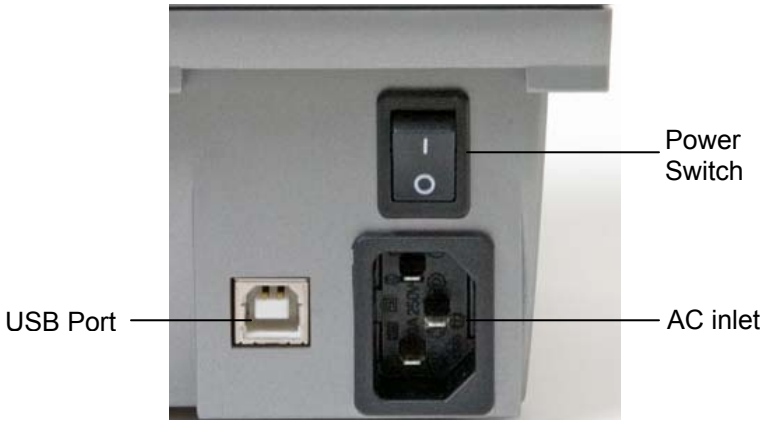
iBlot™ Gel Transfer Device

Front View of iBlot™ Device

The front top view showing various parts of the iBlot™ Gel Transfer Device is shown below.

Rear View of iBlot™ Device

The rear view showing various parts of the iBlot™ Gel Transfer Device is shown below.



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iBlot™ Gel Transfer Device, Continued

Control Panel of iBlot™ Device

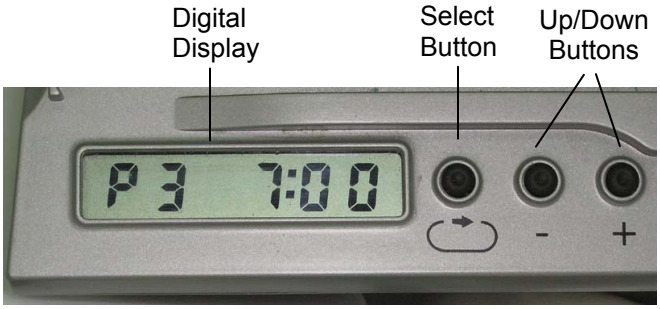
The control panel of the iBlot™ Gel Transfer Device is described below.

The **Digital Display** shows six digits that specify the transfer conditions as follows:

- First two digits indicate the program name
- Remaining four digits specify the time of transfer in minutes and seconds, respectively.

The **Select** Button is used to toggle between program and time.

The **Up/Down** (+/-) Buttons are used to increase or decrease the program, or time. See page 12 for details on selecting the program.



Accessory Products

iBlot™ Gel Transfer Stack

iBlot™ Gel Transfer Stacks are available separately from Invitrogen. Ordering information is provided below.

Product	Quantity	Catalog no.
iBlot™ Gel Transfer Stack, Nitrocellulose, Regular	1 pack of 10	IB3010-01
iBlot™ Gel Transfer Stack, PVDF, Regular	1 pack of 10	IB4010-01
iBlot™ Gel Transfer Stack, Nitrocellulose, Mini	1 pack of 10	IB3010-02
iBlot™ Gel Transfer Stack, PVDF, Mini	1 pack of 10	IB4010-02

Additional Products

Additional reagents that may be used for electrophoresis of proteins are available separately from Invitrogen. Ordering information is provided below. For more information, visit www.invitrogen.com or call Technical Support (page 33).

Product	Quantity	Catalog no.
NuPAGE® Transfer Buffer (20X)	1 L	NP0006-1
NuPAGE® Antioxidant	15 ml	NP0005
WesternBreeze® Chromogenic Kit, Anti-Mouse	1 kit	WB7103
WesternBreeze® Chromogenic Kit Anti-Rabbit	1 kit	WB7105
WesternBreeze® Chemiluminescent Kit, Anti-Mouse	1 kit	WB7104
WesternBreeze® Chemiluminescent Kit, Anti-Rabbit	1 kit	WB7106
Blotting Roller	1	LC2100
SeeBlue® Plus2 Pre-Stained Standard	500 µl	LC5925
MagicMark™ XP Western Protein Standard	250 µl	LC5602
SYPRO® Ruby Protein Blot Stain	200 ml	S-11791

Precast Gels and Premade Buffers

A large variety of precast gels including NuPAGE® Novex®, Tris-Glycine mini and midi gels, and E-PAGE™ gels as well as premade buffers is available from Invitrogen. For details, contact Technical Support (page 33) or visit www.invitrogen.com.

Introduction

Overview

Introduction

The iBlot™ Dry Blotting System consists of the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks that allows you to quickly and reliably perform western blotting of proteins from various types of gels without the need to prepare buffers.

The unique design of the iBlot™ Gel Transfer Device with an integrated power supply, combined with the patented gel matrix technology of the iBlot™ Gel Transfer Stacks generates high field strengths to allow for fast, dry blotting of proteins within 7-8 minutes. There is no need for an external power supply or to prepare buffers, thereby resulting in consistent performance.

The proteins transferred using the iBlot™ Dry Blotting System exhibit higher immunodetection sensitivity when compared to proteins transferred using conventional semi-dry or semi-wet blotting methods.

See next page to understand how the iBlot™ Dry Blotting System works and page 4 for details on various parts of the system.

System Components

The iBlot™ Dry Blotting System consists of:

- iBlot™ Gel Transfer Device

  The iBlot™ Gel Transfer Device is a self-contained blotting unit with an integrated power supply that allows for fast, dry blotting of proteins. See page 4 for details.

- iBlot™ Gel Transfer Stacks

  The iBlot™ Gel Transfer Stacks are disposable stacks with an integrated nitrocellulose or PVDF transfer membrane to perform dry blotting of proteins. Each iBlot™ Gel Transfer Stack contains a copper electrode and appropriate cathode and anode buffers in the gel matrix to allow fast, reliable dry blotting of proteins without the need to prepare buffers. See page 6 for details.

Features

Important features of the iBlot™ Dry Blotting System are listed below:

- User-friendly iBlot™ Gel Transfer Device design with an integrated power supply for fast, reliable protein transfer within 7 minutes
- Ability to perform blotting of E-PAGE™, mini, and midi gels
- Unique, iBlot™ Gel Transfer Stacks with integrated nitrocellulose or PVDF transfer membrane allow dry electroblotting of proteins without the need to prepare buffers, and are compatible for use with NuPAGE® Bis-Tris and Tris-Acetate, Tris-Glycine, Tricine, and E-PAGE™ gels
- Pre-programmed (iBlot™ Gel Transfer Device) with 5 programs for transfer of proteins from various gel types
- Dry blotting enables higher immunodetection sensitivity
- Built-in safety features in the device enhance user safety

Continued on next page

System Overview

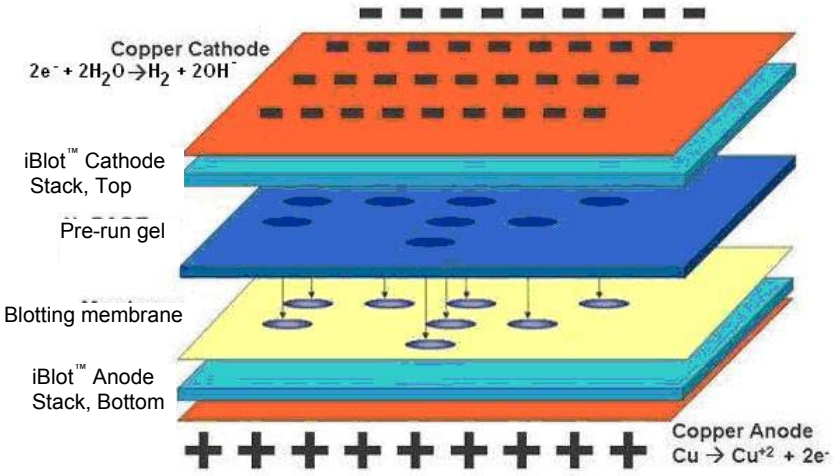
The iBlot™ Dry Blotting System is based on the dry blotting concept which utilizes the unique, patented gel matrix technology developed by Invitrogen for E-Gel® and E-PAGE™ gels.

To use the iBlot™ Dry Blotting System for protein transfer, assemble the iBlot™ Gel Transfer Stacks containing the nitrocellulose or PVDF transfer membrane with your pre-electrophoresed gel on the iBlot™ Gel Transfer Device. Any trapped air bubbles that interfere with efficient protein transfer are removed using the De-bubbling Roller (for E-PAGE™ gels) or using the Blotting Roller (for mini or midi gels). The blotting is performed using a specific program.

The following features of the iBlot™ Dry Blotting System allow rapid protein transfer without the need for external power supply or premade buffers:

- The iBlot™ Gel Transfer Stacks act as ion reservoirs that contain the appropriate anode and cathode buffers incorporated into the gel matrix, eliminating the need for premade buffers or soaked filter papers, and minimizing handling resulting in consistent performance. The iBlot™ Gel Transfer Stacks also contain the copper electrodes (anode and cathode) required for electrophoresis. The protein transfer consistency is increased since the copper anode does not generate oxygen gas as a result of water electrolysis, as compared to conventional inert electrodes present in other blotting systems. See figure below.
- The design of the iBlot™ Gel Transfer Device reduces the distance between electrodes and the integrated power supply. This unique design combined with the gel matrix technology of the iBlot™ Gel Transfer Stacks allows the system to generate high field strength and high protein currents increasing the transfer speed.

Schematic of iBlot™ Dry Blotting System showing the flow of current



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Transfer Membrane

The iBlot™ Gel Transfer Stacks are assembled with the transfer membrane and are available with:

- Nitrocellulose membrane (0.2 µm)

  The nitrocellulose membrane is composed of 100% pure nitrocellulose to provide high-quality transfer. The membrane is compatible with commonly used detection methods such as staining, immunodetection, fluorescence, or radiolabeling but not recommended for reprobing. The proteins bind to the membrane due to hydrophobic and electrostatic interactions. The protein binding capacity is 209 µg/cm^2.

- PVDF membrane (0.2 µm, low fluorescence)

  The PVDF membrane has higher binding capacity than nitrocellulose and is physically stronger than nitrocellulose allowing reprobing of proteins. **The PVDF membrane is preactivated and ready for use without any pretreatment with alcohols.** The membrane is compatible with commonly used detection methods such as staining, immunodetection, fluorescence, or radiolabeling. The proteins bind to the membrane due to hydrophobic interactions. The protein binding capacity is 240 µg/cm^2.

Purpose of the Manual

This manual provides the following information:

- Overview of the dry blotting process to transfer proteins
- Details and specifications on the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks
- Protocol to perform blotting using the iBlot™ Gel Transfer Device with the De-bubbling Roller or Blotting Roller
- Disassembling the iBlot™ Gel Transfer Device
- Tips on optimizing blotting
- Examples of expected results
- Troubleshooting

Note: Immunodetection protocols are not included in this manual.

Downloading Upgrades

Upgrades for the iBlot™ Device firmware are available. To download iBlot™ Device firmware upgrades, go to www.invitrogen.com/iblot. Follow instructions on the page to download the upgrades.

Description of Parts

Introduction

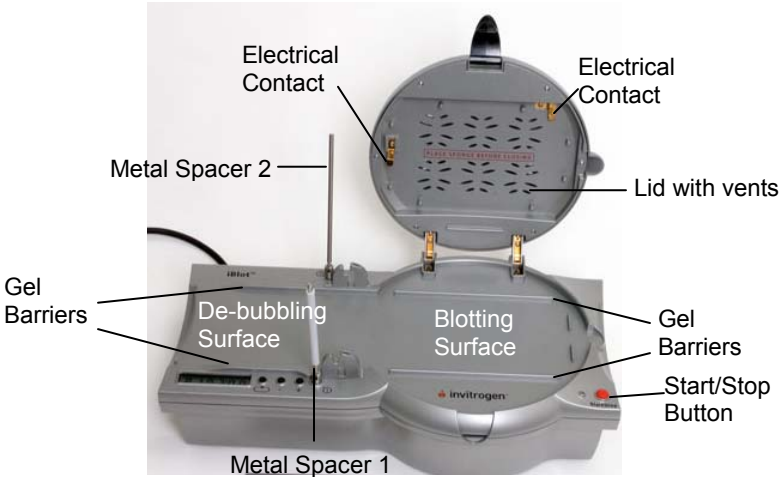
The various parts of the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks are described below.

iBlot™ Gel Transfer Device

The iBlot™ Gel Transfer Device is a blotting device with an integrated power supply capable of producing currents up to 5.5 amp at 25 V. Four printed circuit boards hold the electronic components required to process the systems logic unit, modify voltage and currents for display, and power the blotting process. A pre-installed firmware controls the parameters such as voltage and time, and allows selection of programs (see next page for details on each program).

See page viii for a front and rear view of the device.

A top view of an open iBlot™ Gel Transfer Device identifying various parts is shown below.



Blotting Surface

The blotting surface is the area where the iBlot™ Gel Transfer Stacks are placed with the gel to perform blotting. This area also contains the Gel Barriers that guide the proper placement of the transfer stacks to allow correct electrical contact.

De-Bubbling Surface

The de-bubbling surface is the area where de-bubbling of E-PAGE™ gels is performed using the De-bubbling Roller. This area contains Metal Spacers 1 and 2, and hinges to attach the De-bubbling Roller. Barriers are also present on the de-bubbling surface to guide the proper placement of the iBlot™ Anode Stack, Bottom and the gel to allow efficient de-bubbling. The iBlot™ Anode Stack, Bottom is assembled with the gel, Metal Spacers 1 and 2, the iBlot™ Cathode Stack, Top, and the De-bubbling Roller. The entire assembled transfer stack with the gel is pulled together with the pull tab towards the blotting surface resulting in removal of any trapped air bubbles between the gel and the blotting membrane.

Continued on next page

Description of Parts, Continued

iBlot™ Gel Transfer Device, continued

Lid

The iBlot™ Lid contains ventilation holes to allow for proper ventilation of the unit during the run. The iBlot™ Disposable Sponge (page 7) is placed on the inner side of the iBlot™ Lid within the small protrusions present on the lid that allow proper placement of the sponge. The Lid also contains the electrical contacts for the copper electrodes on the stack to complete the electrical circuit.

Start/Stop Button

The Start/Stop Button is located near the blotting surface and is used to activate the run, stop the run, or reset the program. The red and green status light indicates the status of the run or errors.

Control Panel

The Control Panel is located near the de-bubbling surface and contains the 6-digit digital display, Select button, and Up/Down (+/-) Buttons. See page ix for control panel details.

Programs

The iBlot™ Gel Transfer Device is pre-programmed with 5 voltage programs that allow blotting using a different combination of volts and time. The 5 programs are listed in the table below.

Program	Volt	Default Run Time	Run Time Limit
P1	25	6 minutes	10 minutes
P2	23	6 minutes	11 minutes
P3	20	7 minutes	13 minutes
P4	15	7 minutes	16 minutes
P5	10	7 minutes	25 minutes

The Default Run Time is the default time shown for each program which can be increased or decreased using the Up/Down (+/-) Buttons (see page 12).

The Run Time Limit is the maximum run time that can be programmed for the specific program.

See page 12 to select an appropriate program based on the gel type.

Continued on next page

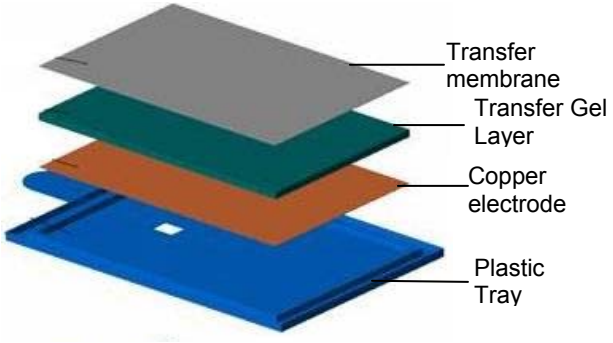
iBlot™ Anode Stack, Bottom

The iBlot™ Anode Stack, Bottom package contains a copper electrode, nitrocellulose (0.2 µm) or PVDF (0.2 µm) membrane, and the Bottom Transfer Gel Layer packaged in a transparent plastic tray. The transparent plastic tray serves as the support for assembling the transfer stacks with the gel and has a tab that assists in the movement of the transfer stack assembly towards the blotting surface during the de-bubbling process. The Bottom Transfer Gel Layer acts as an ion reservoir and is composed of an optimized, proprietary gel composition to provide high-quality transfer of proteins within 7 minutes.

The nitrocellulose (0.2 µm) and PVDF (0.2 µm) membranes **do not** require any pretreatment before use and minimizes protein blow-through during the iBlot™ blotting process.

Always use the iBlot™ Anode Stack, Bottom with the tray in the iBlot™ Device.

See page 7 for iBlot™ Cathode Stack specifications.



The iBlot™ Anode Stack, Bottom is available in standard format for blotting E-PAGE™, midi, or two mini gels (see page vii for dimensions) and Mini format for blotting one mini gel.

Dispose the iBlot™ Anode Stack, Bottom after every use. Do not reuse the iBlot™ Anode Stack, Bottom.

Continued on next page

Description of Parts, Continued

iBlot™ Cathode Stack, Top

The iBlot™ Cathode Stack, Top package contains a copper electrode and the Top Transfer Gel Layer packaged in a red, plastic tray. The Top Transfer Gel Layer acts as an ion reservoir and is composed of an optimized, proprietary gel composition to provide high-quality transfer within 7 minutes.

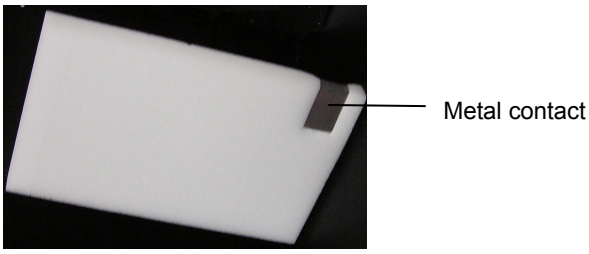
See page vii for iBlot™ Cathode Stack specifications.

The iBlot™ Cathode Stack is available in standard format for blotting E-PAGE™, midi, or two mini gels (see page vii for dimensions) and Mini format for blotting one mini gel.

Dispose the iBlot™ Cathode Stack, Top after every use. Do not reuse the iBlot™ Cathode Stack, Top. Do not use the iBlot™ Cathode Stack, Top with the tray in the iBlot™ Device.

iBlot™ Disposable Sponge

The iBlot™ Disposable Sponge is placed on the inner side of the iBlot™ Lid within the small protrusions on the lid. The iBlot™ Disposable Sponge absorbs any excess liquid on the stacks formed during blotting and generates an even pressure on the stack assembly. See page vii for dimensions of the iBlot™ Disposable Sponge.



The iBlot™ Disposable Sponge is comprised of white melamine and an aluminum metal contact. The metal contact is fixed onto the sponge at a distance of 15 mm from the upper right corner. The metal contact allows proper contact with the electrical contact on the lid as well as the electrode on the assembled iBlot™ Gel Transfer Stacks.

Discard the iBlot™ Disposable Sponge after every use. Do not reuse the iBlot™ Disposable Sponge.

iBlot™ Filter Paper

The iBlot™ Filter Paper is used for blotting mini or midi gels. The iBlot™ Filter Paper is placed on top of the thin gel before placing the iBlot™ Cathode Stack, Top to protect the gel integrity during the blotting process. The iBlot™ Filter Paper is supplied in two sizes (see page vii for dimensions) for efficient blotting of mini and midi gels. **Do not use the iBlot™ Filter Paper for blotting E-PAGE™ gels.**

Note: Failure to use the iBlot™ Filter Paper during blotting of mini or midi gels may result in high currents exceeding the current limit leading to an error (Error2) during the run.

Continued on next page

Description of Parts, Continued

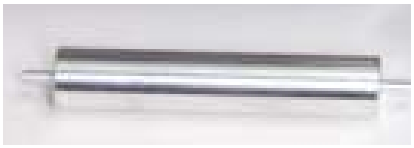
iBlot™ E-PAGE™ Tab

The iBlot™ E-PAGE™ Tab is a steel tab used during blotting of E-PAGE™ gels. The iBlot™ E-PAGE™ Tab is attached to the iBlot™ Cathode Stack, Top and is used to pull the transfer stack assembly towards the blotting surface during the de-bubbling process of E-PAGE™ gels.

De-Bubbling Roller

The De-bubbling Roller is a stainless steel, aluminum roller designed to remove any air bubbles between the gel and blotting membrane during the assembly of the stacks and gel for blotting E-PAGE™ gels. The De-bubbling Roller is installed into the hinges on the de-bubbling surface. The iBlot™ Gel Transfer Stacks and gel are aligned between the Metal Spacers 1 and 2, and the De-bubbling Roller is placed on top. The entire gel assembly is pulled together with the pull tab towards the blotting surface to efficiently remove any air bubbles. Use the protocol on page 13 to perform blotting using the De-Bubbling Roller.

Do not use the De-bubbling Roller for mini, midi, or gels other than E-PAGE™ gels as these gels may stretch and tear.



Blotting Roller

The Blotting Roller is a Delrin roller attached to a stainless steel handle (8.6 cm wide). The Blotting Roller is used to remove any air bubbles between the gel and blotting membrane during the assembly of the stacks and gel. Use the protocol on page 18 to perform blotting of gels using the Blotting Roller.

Experimental Overview

Experimental Outline

The table below outlines the experimental steps necessary to perform western blotting using the iBlot™ Gel Transfer Device. For more details on each step, see indicated pages.

Step	Action	Page
1	Remove the gel from the gel cassette.	13, 18
2	Assemble the iBlot™ Gel Transfer Device with the iBlot™ Gel Transfer Stacks and your protein gel using:	
	• De-bubbling Roller	14
	• Blotting Roller	19
3	Perform western blotting using the recommended parameters.	21
4	Disassemble the iBlot™ Gel Transfer Device.	24

Materials Needed

You need the following items. Ordering information is on page x.

- iBlot™ Gel Transfer Stack for blotting E-PAGE™, Novex® Midi Gels, or two mini gels (see page 10 for recommended gel types)
- iBlot™ Gel Transfer Stacks, Mini for blotting one mini gel
- Prerun gel containing protein samples and protein standards

Methods

General Guidelines

Introduction

General guidelines for using the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks are discussed below.

Recommended Gel Types

The gel types compatible for use with iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks are listed below.

Note: The iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stack is **not** yet optimized for Northern or Southern blotting with agarose gels (E-Gel®) or TBE polyacrylamide gels.

Gel Type	Size	iBlot™ Gel Transfer Stack
E-PAGE™ *48* or *96* Gels	13.5 cm (l) × 10.8 cm (w) 3.7 mm thick	iBlot™ Gel Transfer Stack, Regular
Midi Gels (NuPAGE® Novex® Bis-Tris, Tris-Acetate, or Tris-Glycine Midi Gels, or equivalent gels)	13 cm (l) x 8.3 cm (w) 1.0 mm thick	iBlot™ Gel Transfer Stack, Regular
Mini Gels (NuPAGE® Bis-Tris or Tris-Acetate, Tricine, Tris-Glycine Gels or equivalent gels)	8 cm (l) x 8 cm (w) 1.0 and 1.5 mm thick	iBlot™ Gel Transfer Stack, Regular **or** iBlot™ Gel Transfer Stack, Mini

Recommended Parameters

Use the following parameters for blotting based on the gel type that you use.

Gel Type	Program	Volts	Run Time
E-PAGE™ *48*	P3	20	7-8 minutes
E-PAGE™ *96*	P3	20	7-8 minutes
Novex® Midi Gel, 1 mm thick	P3	20	7-8 minutes
2 Mini Gels (1.0 or 1.5 mm thick)	P3	20	7-8 minutes
1 Mini Gel (1.0 or 1.5 mm thick) using **mini** transfer stacks	P3	20	7-8 minutes

Custom parameters are also easily created using a combination of programs (P1-P5) and time (up to the time limit listed for each program) for gel types not listed above.

Continued on next page

Recommended Protocols

Use the following recommended blotting protocols based on the gel type that you use:

- For E-PAGE™ gels, use the blotting protocol with the De-bubbling Roller described on page 13
- For mini or midi gels, use the blotting protocol with the Blotting Roller described on page 18

To obtain the best results, follow these recommendations:

- Wear gloves at all times during the entire blotting procedure to prevent contamination of gels and membranes.
- Do not touch the membrane or gel with bare or gloved hands. This may contaminate the gel or membrane and interfere with further analysis. If you need to adjust the membrane, always use forceps.
- Use the appropriate gel type and iBlot™ Gel Transfer Stacks as described on the previous page.
- Avoid using expired iBlot™ Gel Transfer Stacks. Always use the transfer stacks before the specified expiration date printed on the package.
- Remove air bubbles as indicated in the protocol using the De-bubbler Roller or Blotting Roller supplied with the device.
- Do not trim the membrane or iBlot™ Gel Transfer Stacks to fit your gel size. See previous page for gel sizes that are compatible with iBlot™ Device. Note that iBlot™ Gel Transfer Stacks, Mini are available for blotting mini gels (page x). Maintain the membrane size identical to the transfer stacks to avoid direct contact between the top and bottom transfer stacks.

Note

We have observed increased immunodetection sensitivity with the iBlot™ Dry Blotting System. If you are using the iBlot™ system for the first time, you may need to load less protein, use more diluted antibody for detection, or perform detection for a shorter time as compared to traditional semi-dry or wet blotting systems. You may also need to optimize the immunodetection based on your initial results.

Getting Started

Installing the iBlot™ Gel Transfer Device

1. **Check the Power Cord supplied with the unit to ensure that the cord is compatible with the local socket format.**
2. Place the iBlot™ Gel Transfer Device on a level laboratory bench. Keep the area around the device clear to ensure proper ventilation of the unit.
3. **For your safety:** Position the device properly such that the **power** switch and the AC inlet located on the rear of the unit (page viii) are easily accessible.
4. Ensure the AC power switch is in the **Off** position (page viii).
5. Attach the power cord to the AC inlet and then to the electrical outlet. Use only properly grounded AC outlets and power cords.

You are ready to use the iBlot™ Gel Transfer Device for blotting applications. See pages 13-18 for the blotting procedure.

Using the iBlot™ Device for the First Time

If you are using the iBlot™ Gel Transfer Device for the first time, you may wish to clean the Metal Spacers 1 and 2, De-bubbling Roller, and blotting surface with a damp cloth before use. Allow the parts to dry before blotting.

Selecting a Program

You need to select an appropriate program on the iBlot™ Device prior to assembling the device with iBlot™ Gel Transfer Stacks and your gel.

1. When the electrophoresis of your samples is almost complete, press the power switch (located on the rear of the device, page viii) to turn **ON** the iBlot™ Gel Transfer Device.

   The fan in the device begins to run and digital display shows text which is stabilized in few seconds to display the default parameters (P 3.0 7:00) or last program used.
2. Select the appropriate program based on the gel type by pressing the Select button to toggle between program, minutes, and seconds. Once the selected item blinks, use the Up/Down (+/-) Buttons for changing the values to the desired parameters as shown below:

Gel Type	Program	Volts	Run Time
E-PAGE™ *48*	P3	20	7-8 minutes
E-PAGE™ *96*	P3	20	7-8 minutes
Novex® Midi Gel, 1 mm thick	P3	20	7-8 minutes
2 Mini Gels (1.0 or 1.5 mm thick)	P3	20	7-8 minutes
1 Mini Gel (1.0 or 1.5 mm thick) using **mini** transfer stacks	P3	20	7-8 minutes

   Custom parameters are also easily created using a combination of programs (P1-P5) and time (up to the time limit listed for each gel type, page 5) for a specific gel type not listed above.

Note: You may need to optimize the blotting parameters (volts or time) based on your initial results. See page 25 for optimizing blotting conditions.

Note

The maximum voltage and current of the output to gel stacks is 25 VDC and 5.5 Amp.

Using the iBlot™ Device with the De-Bubbling Roller

Introduction

Instructions are provided in this section to assemble the iBlot™ Gel Transfer Device with the De-Bubbling Roller for blotting E-PAGE™ Gels.

If you wish to blot mini, midi, or other gels, see page 18 for the blotting protocol.

Materials Needed

You will need the following items:

- Pre-run E-PAGE™ gel or equivalent containing your protein samples and standards
- iBlot™ Gel Transfer Stacks (page x)

Removing the Gel

Remove the gel from the cassette for transfer after completion of electrophoresis as described below.

Open the E-PAGE™ cassette using the red plastic Butterfly Opener supplied with the gel to remove the E-PAGE™ gel. For details, refer to the E-PAGE™ manual supplied with the gel.

Note

- There is no need for any pretreatment of the gel after electrophoresis. Wash the E-PAGE™ gel briefly in deionized water to remove any small gel pieces attached to the gel.
- The transfer membrane is supplied in a ready-to-use format in the stacks without any need for pretreatment. Do not treat the PVDF membrane with methanol as the PVDF membrane is preactivated prior to assembly with the transfer stack.
- To obtain the best blotting results with the E-PAGE™ gels, we recommend that you use the De-bubbling Roller. However, you may use the Blotting Roller for de-bubbling E-PAGE™ gels as described on page 18.

Continued on next page

Using the iBlot™ Device with the De-Bubbling Roller, Continued

Assembling the iBlot™ Device

Instructions are provided below to assemble the iBlot™ Gel Transfer Device with iBlot™ Gel Transfer Stacks and E-PAGE™ precast gels. See page 18 for blotting mini, midi, or other gels.

1. Open the lid of the device and pull up the Metal Spacers 1 and 2. If you have attached the De-bubbling Roller to the device, then remove the roller as shown in the figure below.

2. Remove the package labeled iBlot™ Anode Stack, Bottom from the iBlot™ Gel Transfer Stacks Box. Remove the laminated sealing of the iBlot™ Anode Stack, Bottom and **keep the stack in the transparent plastic tray.**

3. Place the iBlot™ Anode Stack, Bottom stack **with the tray** to the left of the blotting surface area such that the tab on the tray is on the right side of the De-bubbling Roller, as shown below. Slide the bottom stack to the left until the stack is blocked by the Gel Barriers present on the left side of the device.

   Note: Always handle the iBlot™ Anode Stack, Bottom using the plastic tray without disturbing the gel and membrane layers in the stack. **Do not** touch the transfer membrane on the stack.

Continued on next page

Assembling the iBlot™ Device, continued

4. Clean the Metal Spacer 1 with a damp cloth or tissue and place the spacer on the membrane as shown below.

5. Place the prerun gel containing your protein samples on Metal Spacer 1 such that the gel is aligned to the lower right corner of the bottom stack with the wells of the E-PAGE™ gel facing up.

6. Clean the Metal Spacer 2 with a damp cloth or tissue and place the spacer over the gel as shown below.

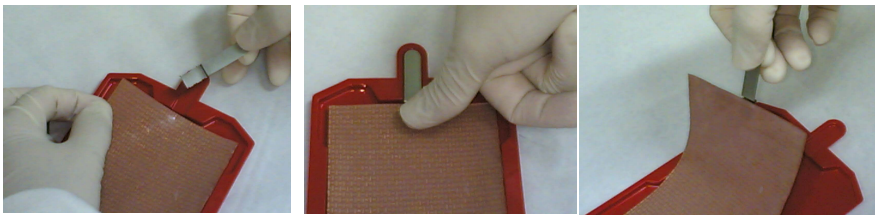
7. Remove the package labeled iBlot™ Cathode Stack, Top from the iBlot™ Gel Transfer Stacks Box. Remove the iBlot™ Cathode Stack, Top from the package.

Continued on next page

Assembling the iBlot™ Device, continued

8. Insert the steel iBlot™ E-PAGE™ Tab in the plastic tray groove with the tab teeth facing up (figure A). Gently press the iBlot™ Cathode Stack over the teeth to allow the teeth to penetrate into the copper electrode (figure B). Remove the iBlot™ Cathode Stack, Top from the red plastic tray using the iBlot™ E-PAGE™ Tab (figure C).

A **B** **C**



9. Place the iBlot™ Cathode Stack, Top **without the tray** on top of Metal Spacer 2 with the copper electrode side facing up. Ensure that all layers are aligned to the right to perform efficient de-bubbling.

10. Insert the De-bubbling Roller into the two grooves and lower the roller to its lowest location while holding the pull tab. The resulting assembly consists of the gel, and cathode and anode stacks placed between two Metal Spacers 1 and 2 with the De-bubbling Roller on top of the assembly as shown below.

Continued on next page

Assembling the iBlot™ Device, continued

11. Hold the iBlot™ E-PAGE™ Tab and plastic tab on the iBlot™ Anode Stack, Bottom together and pull the assembly (anode and cathode stacks, and gel) together through the De-bubbling Roller towards the blotting surface, in one smooth, uninterrupted movement until the assembly reaches the Gel Barriers on the blotting surface (figure A). At the end of de-bubbling, all layers are aligned to the right as shown below (figure B).

A **B**

12. Place the iBlot™ Disposable Sponge on the inner side of the lid (between the small protrusions on the lid that hold the sponge in its place) such that the metal contact is to the top right.

The sponge absorbs any excess liquid generated during blotting and exerts an even pressure on the stack surface.

Performing Blotting

After assembling the iBlot™ Gel Transfer Device, perform blotting within 15 minutes of assembling the stacks with the gel as described below.

1. Close the iBlot™ Lid and secure the latch. The red light is on indicating a closed circuit. Ensure the correct program is selected (page 12).
2. Press the Start/Stop button to start the transfer. The red status light changes to green. The transfer continues using the programmed parameters.
3. At the end of the transfer, current automatically shuts off and the iBlot™ Gel Transfer Device signals the end of transfer with repeated beeping sounds, and flashing red light and digital display.

   Note: Previous versions of the iBlot™ Gel Transfer Device (firmware versions prior to 2.7.9), signaled the end of transfer with repeated beeping sounds, and flashing **green** light (instead of red light) and digital display.
4. Press and release the Start/Stop button to stop the beeping. The light turns to a steady red light.
5. Proceed to **Disassembling the iBlot™ Gel Transfer Device**, page 24.

Using the iBlot™ Device with the Blotting Roller

Introduction

Instructions are provided in this section to assemble the iBlot™ Gel Transfer Device without the De-Bubbling Roller for blotting mini, midi, or other gels.

If you wish to blot E-PAGE™ gels, see page 13 for the blotting protocol.

Materials Needed

You will need the following items:

- Prerun mini or midi gel containing your protein samples and standards
- iBlot™ Gel Transfer Stacks for blotting one midi gel or two mini-gels (page x)
- iBlot™ Gel Transfer Stacks, Mini for blotting one mini gel (page x)
- Blotting Roller supplied with the device

Removing the Gel

Remove the gel from the cassette for transfer after completion of electrophoresis as described below.

- Open the mini or midi gel cassette using the Gel Knife by inserting the knife into the narrow gap between the two plates of the cassette. Push up and down gently on the knife's handle to separate the plates. Upon opening the cassette, discard the plate without the gel and slowly remove the gel adhered to the other plate. For details on removing the gel, refer to the manual supplied with the mini or midi gel.
- For other gel types, refer to the manufacturer's recommendations to remove the gel from the cassette.

Note

- There is no need for any pretreatment of the gel after electrophoresis.
- The transfer membrane is supplied in a ready-to-use format in the stacks without any need for pretreatment. Do not treat the PVDF membrane with methanol as the PVDF membrane is preactivated prior to assembly with the transfer stack.
- You may blot E-PAGE™ gels using the blotting protocol with the Blotting Roller. If you wish to use the Blotting Roller for blotting E-PAGE™ gels be sure to:
  - Wash the E-PAGE™ gel briefly in deionized water prior to blotting to remove any small gel pieces attached to the gel.
  - Use the Blotting Roller all over the gel including **all well areas** to obtain efficient blotting.
- If you notice distorted protein bands after using the E-PAGE™ blotting protocol with the Blotting Roller, we recommend that you blot the E-PAGE™ gels using the De-bubbling Roller (page 13).

Continued on next page

Using the iBlot™ Device with the Blotting Roller, Continued

Important

Use the appropriate iBlot™ Gel Transfer Stacks based on the gel that you are blotting. **Do not** trim the membrane or transfer stacks to fit the size of your gel, as the transfer quality is not affected if the prerun gel is smaller than the transfer stack. Always maintain the membrane size identical to the transfer stacks to avoid accidental contact between the iBlot™ Anode and Cathode Stacks.

See page 10 for gel types compatible with the iBlot™ Gel Transfer Device.

- Use the iBlot™ Gel Transfer Stacks, **Regular** for blotting two mini-gels or one midi gel
- Use the iBlot™ Gel Transfer Stacks, **Mini** for blotting one mini gel.

Assembling the iBlot™ Device

Instructions are provided below to assemble the iBlot™ Gel Transfer Device with iBlot™ Gel Transfer Stacks or Mini, and mini, midi, or other gels. See page 13 for blotting E-PAGE™ gels.

1. Open the lid of the iBlot™ Gel Transfer Device. Ensure the blotting surface is clean.
2. Remove the iBlot™ Anode Stack, Bottom (or Mini stack) from the package. Remove the laminated sealing of the iBlot™ Anode Stack, Bottom and **keep the stack in the transparent plastic tray.** Place the iBlot™ Anode Stack, Bottom **with the tray** directly on the blotting surface (under the round lid). Align the anode stack to the Gel barriers on right edge of the blotting surface (see figure below) to avoid accidental contact of the electrical contacts on lid with the iBlot™ Anode Stack, Bottom.

3. Ensure no bubbles are visible between the membrane and the transfer stack gel below the membrane. Remove any trapped air bubbles using the Blotting Roller.

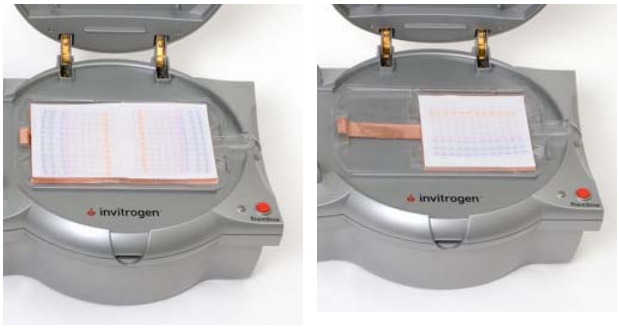
Continued on next page

Using the iBlot™ Device with the Blotting Roller, Continued

Assembling the iBlot™ Device, continued

4. Place the prerun gel on the transfer membrane of the anode stack as described:
   - One midi gel on an iBlot™ Gel Transfer Stack
   - Two mini gels (head-to-head) on an iBlot™ Gel Transfer Stack (figure A)
   - One mini gel on an iBlot™ Gel Transfer Stack, Mini (figure B)

A **B**



5. In a clean container, soak one iBlot™ Filter Paper (or Mini Filter paper based on the gel type used) in deionized water. iBlot™ Filter Paper is included with each iBlot™ Gel Transfer Stacks.
6. Place the presoaked iBlot™ Filter Paper on the prerun gel. Use the Blotting Roller to remove any air bubbles between the membrane and gel as shown below for the Transfer Stack.

   For E-PAGE™ gels, **there is no need** to use a filter paper and be sure to use the Blotting Roller over the well rows to flatten any remaining gel protrusions to ensure even transfer.

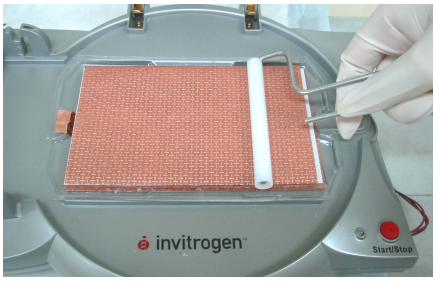
7. Remove the iBlot™ Cathode Stack, Top (or Cathode Stack, Mini) from the package. Discard the red plastic tray.

Continued on next page

Using the iBlot™ Device with the Blotting Roller, Continued

Assembling the iBlot™ Device, continued

8. Place the iBlot™ Cathode Stack, Top (or Cathode Stack, Mini) on top of the presoaked filter paper with the copper electrode side facing up and aligned to the right of the bottom stack. Remove any air-bubbles using the Blotting Roller.



9. Place the iBlot™ Disposable Sponge on the inner side of the lid (between the small protrusions on the lid that hold the sponge in its place) such that the metal contact is to the top right as shown below.

The sponge absorbs any excess liquid generated during blotting and exerts an even pressure on the stack surface.

Performing Blotting

After assembling the iBlot™ Gel Transfer Device, perform blotting as described below. Perform blotting within 15 minutes of assembling the stacks with the gel.

1. Close the iBlot™ Lid and secure the latch. The red light is on indicating a closed circuit. Ensure that the correct program is selected (page 12).
2. Press the Start/Stop button to start the transfer. The red status light changes to green. The transfer continues using the programmed parameters (page 12).
3. At the end of the transfer, current automatically shuts off and the iBlot™ Gel Transfer Device signals the end of transfer with repeated beeping sounds, and flashing red light and digital display.

   Note: Previous versions of the iBlot™ Gel Transfer Device (firmware versions prior to 2.7.9), signaled the end of transfer with repeated beeping sounds, and flashing **green** light (instead of red light) and digital display.
4. Press and release the Start/Stop button to stop the beeping. The light turns to a steady red light.
5. Proceed to **Disassembling the iBlot™ Gel Transfer Device**, page 24.

iBlot™ Quick Reference Guide

Introduction

A quick reference guide for operating the iBlot™ Gel Transfer Device is provided below.

Mode	Action	Sound	Light	Display
iBlot™ plugged in	iBlot™ connected to an electrical outlet and power switch is on	--	--	Version of iBlot™ firmware
iBlot™ turned on	No transfer stacks detected with lid opened	--	--	Default setting or the last defined program/time appear
Program selection	Press and release the Select Button	--	--	Program name blinks
Time selection (minutes)	Press the Select Button and use the Up and Down Buttons (+/-) to change values	--	--	Minutes blink
Time selection (seconds)	Press the Select Button and use the Up and Down Buttons (+/-) to change values	--	--	Seconds blink
Ready to run	Transfer stacks placed in the device and lid closed	--	Steady red	Program and time
Run	Press and release the Start/Stop button	--	Steady green	Count down time
Running error alert	Open the lid and fix the error (lost contact with stacks or short circuit)	Continuous beeping	Flashing red	Error1, Error2, or Error3*
Error fixed	Close the lid	Continuous beeping	Flashing green	Error1, Error2, or Error3*
Continue after error	Press and release the Start/Stop button	--	Steady green	Count down time
Restart after error	Press and hold the Start/Stop button • Transfer stacks placed in the device and lid closed • No transfer stacks detected with lid opened	--	• Steady red --	• Program and time • Default setting or the last defined program/time appear

*Error 3 is displayed in iBlot™ Gel Transfer Device with firmware version 2.8.1 and above.

Continued on next page

Mode	Action	Sound	Light	Display
End of run	Automatic	Continuous beeping for 2 minutes (or less if Start/Stop button is pressed) followed by a single beep every minute	Flashing red	Program and time
Run ends after an external power failure	Transfer stacks placed in the device and lid closed	--	Steady red	Program and time

Disassembling the iBlot™ Gel Transfer Device

Introduction

Refer to the instructions below to disassemble the iBlot™ Gel Transfer Device.

Procedure

To obtain good transfer and detection results, disassemble the device and stacks within 30 minutes of ending the blotting procedure.

1. Open the lid of the iBlot™ Device.
2. Remove the iBlot™ E-PAGE™ Tab (used for blotting E-PAGE™ gels only). Rinse the tab with deionized water and store in a dry place for future use. **Do not discard the iBlot™ E-PAGE™ Tab.**
3. Discard the iBlot™ Disposable Sponge and iBlot™ Cathode Stack, Top.
4. Carefully remove and discard the gel and filter paper (if used) as shown below. Remove the transfer membrane from the stack and proceed with the blocking procedure or stain the membrane (see next page for details).

   Note: If you are using PVDF membranes, place the membrane immediately into the blocking or staining solution (or water) as PVDF membranes dry quickly. If the PVDF membrane is dried, rewet the membrane with methanol and rinse with deionized water a few times before use.

5. Discard the iBlot™ Anode stack, Bottom.
6. At this point, the iBlot™ Gel Transfer Device is ready for another run (no cooling period is required). If you are not using the device, turn off the power switch located on the back of the iBlot™ Gel Transfer Device.

Important

Do not reuse the iBlot™ Disposable Sponge, iBlot™ Filter Paper, and iBlot™ Cathode and Anode Stacks after blotting. Discard after each use.

Cleaning and Maintenance

Clean the blotting surface, Metal Spacers 1 and 2, and the De-bubbling Roller with a damp cloth or paper tissue. Allow the parts to dry before use.

For any other repairs and service, contact Technical Support (page 33). Do not perform any repairs or service on the iBlot™ Gel Transfer device to avoid damaging the iBlot™ Device.

Post Transfer Analysis and Optimizing Blotting

Post Transfer Analysis

After the transfer, you may proceed to immunodetection, store the membrane for future use, or stain the membrane.

- For immunodetection of proteins, use the WesternBreeze® Chromogenic or Chemiluminescent Immunodetection Kits available from Invitrogen (page x) or any other immunodetection kit.
- For storing nitrocellulose membranes, air dry the membrane and store the membrane in an air-tight plastic bag at room temperature or 4°C. Avoid storing nitrocellulose below -20°C, as they turn brittle.
- For storing PVDF membranes, air dry the membrane and store the membrane in a air-tight plastic bag at room temperature, 4°C, or -80°C. When you are ready to use the membrane, rewet the membrane with methanol for a few seconds, followed by thorough rinsing of the membrane with deionized water to remove methanol.
- For staining membranes after blotting, you may use any total protein membrane staining methods such as Coomassie® Blue R-250, Ponceau S, Amido Black, or SYPRO® Ruby Blot Stain (page x). The iBlot™ Gel Transfer Device blotting protocol is compatible with most total protein membrane staining methods listed above.

  Note: The sensitivity of total protein membrane staining after using the dry blotting protocol with iBlot™ Gel Transfer Device is slightly lower than the total membrane protein staining obtained with the semi-wet transfer protocol. However, due to the nature of dry blotting, lower transfer does not affect the immunodetection sensitivity.

If you do not detect any proteins on the membrane after immunodetection or staining, refer to Troubleshooting on page 29. **Refer to the manufacturer's recommendations for optimizing immunodetection.**

Optimizing Blotting

When using the iBlot™ Gel Transfer Device, most proteins transfer efficiently using the protocol in this manual. Based on specific properties of a protein or a set of proteins, some optimization of the blotting protocol may be necessary.

Perform optimization of blotting as follows:

- Performing an equilibration step prior to transfer

  To improve the transfer of high-molecular weight proteins from mini or midi NuPAGE® or Tris-Glycine gels, equilibrate the gel in 100 ml Equilibration Buffer (2X NuPAGE® Transfer Buffer containing 10% methanol and 1:1000 NuPAGE® Antioxidant) for 20 minutes at room temperature on a shaker prior to transfer. After equilibration, use the gel for transfer using the iBlot™ Device as described in this manual.

  Note: The equilibration step improves the transfer of high molecular weight proteins but may increase the blow through of low molecular weight proteins. **Do not** use this equilibration step with E-PAGE™ gels as no improvement in the transfer is observed.

- Increasing or decreasing the transfer time

  Based on the initial results, you can increase or decrease the transfer time using the Up/Down buttons in 30-second increments. **Do not** perform transfer for more than the time limit indicated for each program (page 5).

Continued on next page

Post Transfer Analysis and Optimizing Blotting, Continued

Note

- It is normal for some proteins to remain in the gel as some high molecular weight proteins do not transfer completely using the iBlot™ Gel Transfer Device as compared to wet transfer apparatus.
- Since the sensitivity of detection using the iBlot™ Gel Transfer Device is higher as compared to semi-wet and semi-dry blotting, complete transfer of proteins is not required.
- Almost complete transfer of prestained standard protein bands is observed with the iBlot™ Gel Transfer Device. However, note that the complete transfer of prestained protein standards does not indicate complete transfer of other proteins.

Examples of Results

Introduction

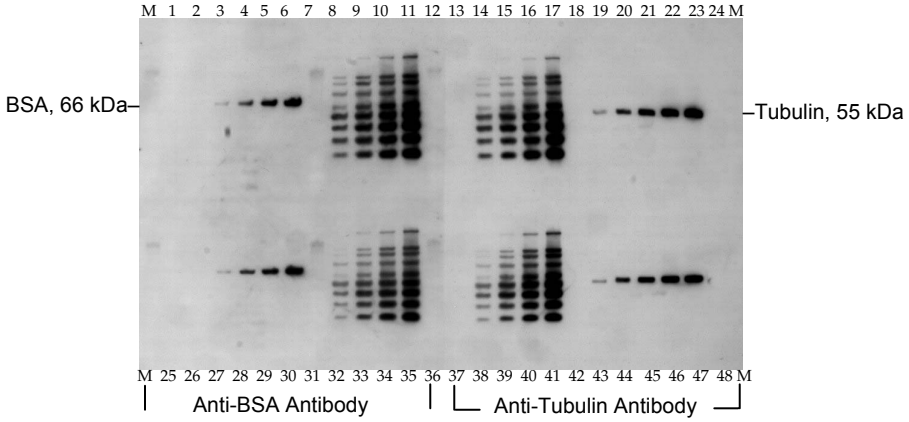
Examples of results obtained using the iBlot™ Dry Blotting System are shown below.

E-PAGE™ Gel Results Using Nitrocellulose

E-PAGE™ *48* 8% Gel was subjected to blotting using the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks with the De-bubbling Roller as described in this manual. The proteins on the nitrocellulose membrane were detected using the WesternBreeze® Chemiluminescent Anti- Mouse Kit (page x) using 1:10,000 dilution of anti-BSA antibody (left panel) or 1:10,000 dilution of anti-tubulin antibody (right panel).

The gel contains the following samples (rows not indicated are blank):

Lane	Sample
2, 3, 4, 5, 6, and 26, 27, 28, 29, 30	BSA (5 ng, 10 ng, 25 ng, 50 ng, and 100 ng)
8, 9, 10, 11, and 14, 15, 16, 17	MagicMark™ XP Western Protein Standard (0.5 µl, 1 µl, 2 µl, and 4 µl)
32, 33, 34, 35, and 38, 39, 40, 41	
19, 20, 21, 22, 23, and 43, 44, 45, 46, 47	Human Colon Cancer cell lysate, SW480 (0.25 µl, 0.5 µl, 1 µl, 2 µl, and 4 µl)

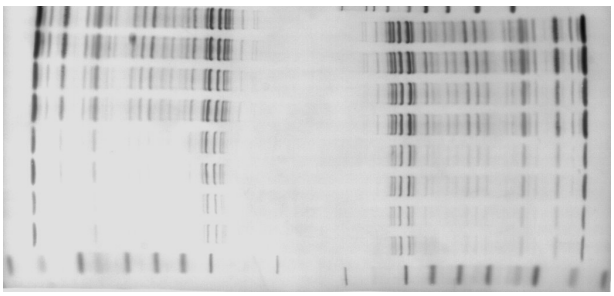


Two Mini Gel Results Using Nitrocellulose

Two NuPAGE® Novex® 4-12% Bis-Tris Mini Gels were subjected to blotting using the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks with the Blotting Roller, as described in this manual. The proteins on the nitrocellulose membrane were detected using the WesternBreeze® Chromogenic Anti-Rabbit Kit (page x) using 1:2,000 dilution of an anti-*E. coli* antibody.

Lane 1: 5 µl SeeBlue® Plus2 Pre-Stained Protein Standard

Lanes 2-9: Duplicate samples of *E. coli* lysate diluted 1:16 (0.5 µl, 1 µl, 2 µl, 4 µl, respectively)

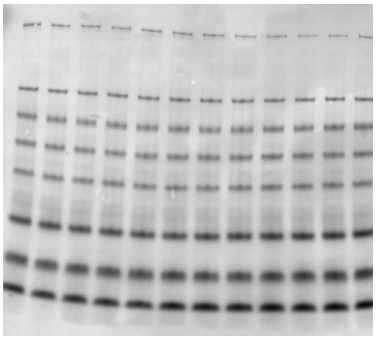


Expected Results, Continued

Mini Gel Results Using Nitrocellulose

SeeBlue® Plus2 Pre-Stained Protein Standard (5 µl) was electrophoresed on a NuPAGE® Novex® 4-12% Bis-Tris Mini Gel. After electrophoresis, the gel was subjected to blotting using the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks, Mini with Blotting Roller as described in this manual.

The figure below shows good transfer of protein standard bands on to the nitrocellulose membrane.



Mini Gel Results Using PVDF

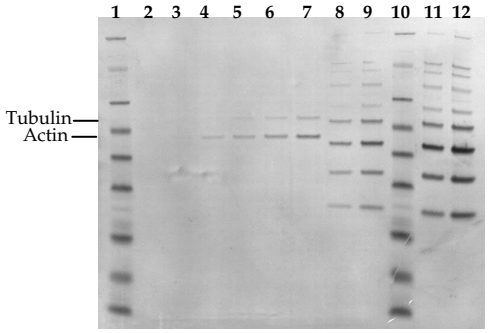
The NuPAGE® Novex® 4-12% Bis-Tris Mini Gel was subjected to blotting using the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks with the Blotting Roller, as described in this manual. The proteins on the PVDF membrane were detected using the WesternBreeze® Chromogenic Anti-Mouse Kit (page x) using 1:10,000 dilution of an anti-tubulin and 1:5,000 anti-actin antibody.

Samples on the gel:

Lanes 1, 10: 5 µl SeeBlue® Plus2 Pre-Stained Protein Standard

Lanes 2-7: SW480 lysate, 0.5 µg/µl (0.125 µl, 0.25 µl, 0.5 µl, 1 µl, 2 µl, 4 µl, respectively)

Lanes 8, 9, 11, 12: MagicMark™ XP Western Protein Standard (0.5 µl, 1 µl, 2 µl, 4 µl, respectively)



Troubleshooting

Introduction

Review the information below to troubleshoot your experiments using the iBlot™ Gel Transfer Device and iBlot™ Gel Transfer Stacks.

To troubleshoot immunodetection, refer to the manufacturer's recommendations for optimizing immunodetection.

Problem	Cause	Solution
No current (red light is not on after securing the lid)	Incomplete electric circuit due to:	
	• iBlot™ Disposable Sponge covers the metal contact or the metal contact on the sponge is on the left	• Reinsert the iBlot™ Disposable Sponge such that the metal contact on the sponge is on the top right of the lid and is in contact with the electrode on the transfer stack (page 17).
	• Incorrect position of the transfer stacks or improper assembly of the transfer stacks	• Make sure the transfer stack is placed in the proper position in the blotting surface to allow proper contacts with the electrodes. Ensure the transfer stacks are assembled correctly; use the iBlot™ Anode Stack, Bottom first followed by the gel and iBlot™ Cathode Stack, Top.
	• Incorrect position of the pull tab	• Ensure the pull tab from the iBlot™ Cathode Stack, Top is towards the right of the assembly in the blotting surface (page 17).
	• iBlot™ Anode Stack, Bottom placed on the device **without** the tray including the electrical contact	• **Do not** remove the iBlot™ Anode Stack, Bottom from the tray during the assembly. The blotting is performed with the bottom stack in the plastic tray.
	• iBlot™ Cathode Stack, Top placed on the device **with** the tray	• Always remove the iBlot™ Cathode Stack, Top from the red plastic tray before placing the top stack on the assembly. Do not use the iBlot™ Cathode Stack, Top with the tray.
	• The metal safety contacts in the lid hinge may be dirty and do not make contact	• Clean the metal safety contacts in the lid hinge with a cotton swab and water.
Digital display shows Error1 indicating an open electrical circuit during the run	The lid opened during the run	Close the lid. Continue the run by briefly pressing Start/Stop button or restart the run by pressing and holding the Start/Stop button.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Digital display shows Error2 indicating a short circuit	• The iBlot™ Cathode Stack, Top is touching the copper electrode on the iBlot™ Anode Stack, Bottom • The layers are not aligned • Current is above 5.5 amp	• Open the lid and align the iBlot™ Cathode Stack, Top to the right. Continue the run by briefly pressing Start/Stop button or restart the run by pressing and holding the Start/Stop button. • Align the layers properly as described in the protocols. Ensure that the electrodes are in contact. • Select a program with a lower voltage. Open the iBlot™ Lid and ensure the stacks are aligned properly. Close the lid and restart the run by subtracting the time already elapsed. • Replace the iBlot™ Gel Transfer Stacks with fresh transfer stacks. Ensure an iBlot™ Filter Paper was used during blotting of mini or midi gels.
Digital display shows Error3 indicating a partial short circuit	The layers are not aligned	Align the layers properly as described in the protocols. Ensure the iBlot™ Anode Stack Bottom tray is aligned to the Gel barriers on right edge of the blotting surface to avoid any accidental contact of the lid electrical contacts with the iBlot™ Anode Stack, Bottom.
Corrosion of the iBlot™ Cathode Stack, Top	Incorrect placement of the top stack	Be sure the iBlot™ Cathode Stack, Top is placed correctly with the copper electrode side facing up. Avoid placing the top stack in the inverted position.
No proteins transferred to the membrane	No current or incorrect program used	See previous page to ensure the electrical circuit is complete and current is flowing through the device. Be sure to use the correct program (page 12).
Empty spots on the membrane	• Presence of air bubbles between the gel and the membrane preventing the transfer of proteins • Expired or creased membranes used	• Be sure to remove all air bubbles between the gel and membrane by using the De-Bubbling Roller for E-PAGE™ Gels or Blotting Roller for other gels. • Use the iBlot™ Gel Transfer Stacks before the expiration date printed on the package.

Continued on next page

Problem	Cause	Solution
Protein bands distorted on membrane (for E-PAGE™ gels)	Non-uniform electric field created around wells	• Ensure that the well protrusions on the E-PAGE™ gel are flattened properly using the De-bubbling Roller or Blotting Roller. • To ensure best blotting results, we recommend using the De-bubbling Roller with E-PAGE™ gels. If you used the Blotting Roller with E-PAGE™ gels, be sure to follow the recommendations on page 18 to obtain good results.

High molecular weight proteins remain in the gel indicated by staining of the gel after transfer	Incorrect program or transfer conditions used
	Note: It is normal for some proteins to remain in the gel as some high molecular weight proteins do not transfer completely using the iBlot™ Gel Transfer Device as compared to semi-wet transfer apparatus.
		• Use the appropriate program and run time based on the gel type as described on page 12.
		• For mini or midi gels:
		  • Use a lower gel percentage to separate the high molecular weight proteins.
		  • Increase the transfer time in 30-second increments.
		  • Perform an equilibration step as described on page 25 to improve transfer.
		• For E-PAGE™ gels:
		  • Increase the transfer time in 30-second increments.
		  • Use P2 program for 8 minutes.

Membrane and the gel turns blue	Longer transfer times result in the deposition of copper ions	Be sure to perform the transfer for the recommended time for each gel type.
iBlot™ Anode Stack, Bottom transfer gel melts to a viscous blue solution	Membrane is trimmed to fit the gel size resulting in direct contact between the iBlot™ Cathode, Top and Anode Bottom stacks	Always maintain the membrane size identical to the transfer stack. Transfer quality is not affected by smaller gel size compared to the membrane.
Signal intensity is similar for different protein loads after detection	High protein load (detection is not within the linear range)	Since the immunodetection sensitivity is higher for dry blotting with iBlot™ device when compared to semi-dry or wet blotting, we recommend that you decrease the protein load, use more diluted antibody, or perform detection for shorter time. You may need to perform some optimization based on your initial results.

Appendix

Explanation of Symbols and Warnings

The iBlot™ Gel Transfer Device complies with the Underwriters Laboratories Inc. regulation, part 15 of the FCC rules, and the European Community Safety requirements. Operation of the iBlot™ Gel Transfer Device is subject to the following conditions:

- Indoor use
- Altitude below 2,000 meters
- Temperature range: 5 to 40ºC
- Maximum relative humidity: 80%
- Installation categories (over voltage categories) II; Pollution degree 2
- Mains supply voltage fluctuations not to exceed 10% of the nominal voltage (100–240 V, 50/60 Hz, 3.3 A).
- Mains plug is a disconnect device and must be easily accessible.
- Do not attempt to open the iBlot™ Gel Transfer Device. To honor the warranty, iBlot™ device can only be opened and serviced by Invitrogen.
- The protection provided by the equipment may be impaired if the equipment is used in a manner not specified by Invitrogen.
- The device must be connected to a mains socket outlet with protective earthing connections.
- Ventilation requirements: room ventilation

The iBlot™ Gel Transfer Device complies with part 15 of the FCC rules. Operation of the device is subject to the following two conditions:

- The device may not cause harmful interference
- The device must accept any interference received, including interference that may cause undesired operation.

Ethrog Biotechnologies Ltd., an Invitrogen company, is the manufacturer and owner of the UL file. For more information, contact:

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Ness-Ziona, Israel 74103

The **Caution** symbol denotes a risk of safety hazard. Refer to accompanying documentation.

WEEE (Waste Electrical and Electronic Equipment) symbol

Technical Support

Web Resources

Visit the Invitrogen Web site at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

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MSDS

MSDSs (Material Safety Data Sheets) are available on our website at www.invitrogen.com/msds.

Product Qualification

Product Qualification

The iBlot™ Gel Transfer Stacks are qualified using the following criteria:

- **Visual Inspection**

  Each component of the iBlot™ Gel Transfer Stack is visually inspected as follows:

  Transfer Gel Layer is inspected for size, integrity, uniformity, absence of bubbles and spots, and must meet the set specifications.

  Copper Electrodes must be clean without any blue or other spots.

  Plastic Tray must be clean without any deformations and the electrode contact attached to the bottom plastic tray must be proper without showing any sign of corrosion.

  Nitrocellulose and PVDF Membrane must be clean and free of spots or bubbles, and must not be dry.

- **Functional Test**

  The iBlot™ Dry Blotting System is functionally qualified by blotting suitable E-PAGE™ and NuPAGE® gels as described in this manual using pre-stained protein standards and protein samples. The system is tested for initial and final current on the device, detection sensitivity, and the presence of bubbles, smears, distortions after blotting on the membrane, and must meet the set specifications.

Purchaser Notification

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Warranty

iBlot™ Gel Transfer Device

Invitrogen warrants that this product will be free from defects in material and workmanship for a period of one (1) year from date of purchase. If a defect is present, Invitrogen will, at its option, repair, replace, or refund the purchase price of this product at no charge to you, provided it is returned during the warranty period. This warranty does not apply if the product has been damaged by accident, abuse, misuse, or misapplication, or from ordinary wear and tear. For your protection, items being returned must be insured against possible damage or loss. This warranty shall be limited to the replacement of defective products. **It is expressly agreed that this warranty will be in lieu of all warranties of fitness and in lieu of the warranty of merchantability.**

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